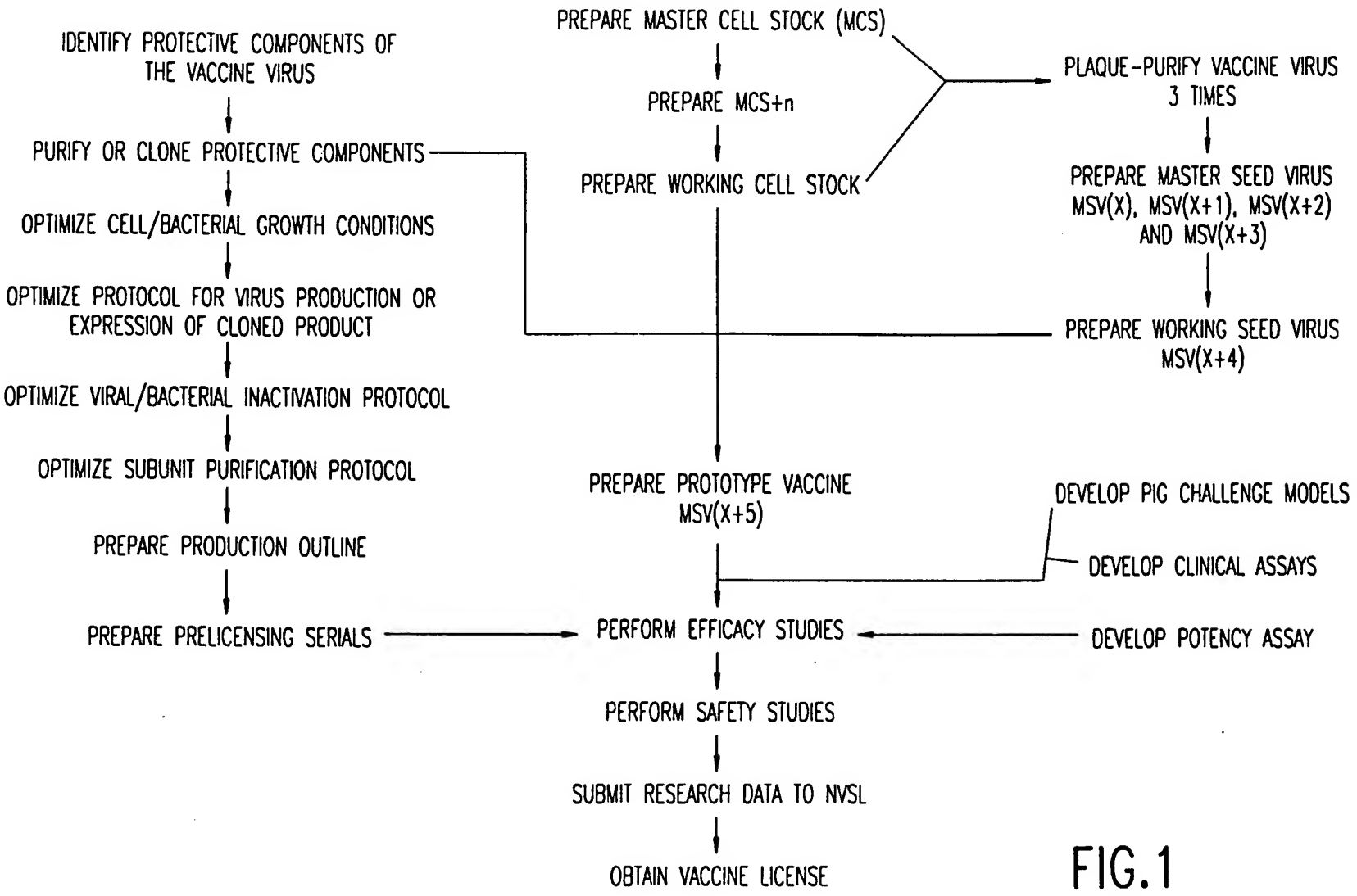

IDENTIFY PROTECTIVE COMPONENTS OF THE VACCINE VIRUS
PURIFY OR CLONE PROTECTIVE COMPONENTS
OPTIMIZE CELL/BACTERIAL GROWTH CONDITIONS
OPTIMIZE PROTOCOL FOR VIRUS PRODUCTION OR EXPRESSION OF CLONED PRODUCT
OPTIMIZE VIRAL/BACTERIAL INACTIVATION PROTOCOL
OPTIMIZE SUBUNIT PURIFICATION PROTOCOL
PREPARE PRODUCTION OUTLINE
PREPARE PRELICENSING SERIALS
PREPARE MASTER CELL STOCK (MCS)
PREPARE MCS+n
PREPARE WORKING CELL STOCK
PLAQUE-PURIFY VACCINE VIRUS 3 TIMES
PREPARE MASTER SEED VIRUS MSV(X), MSV(X+1), MSV(X+2) AND MSV(X+3)
PREPARE WORKING SEED VIRUS MSV(X+4)
PREPARE PROTOTYPE VACCINE MSV(X+5)
DEVELOP PIG CHALLENGE MODELS
DEVELOP CLINICAL ASSAYS
PERFORM EFFICACY STUDIES
DEVELOP POTENCY ASSAY
PERFORM SAFETY STUDIES
SUBMIT RESEARCH DATA TO NVSL
OBTAIN VACCINE LICENSE

FIG.1

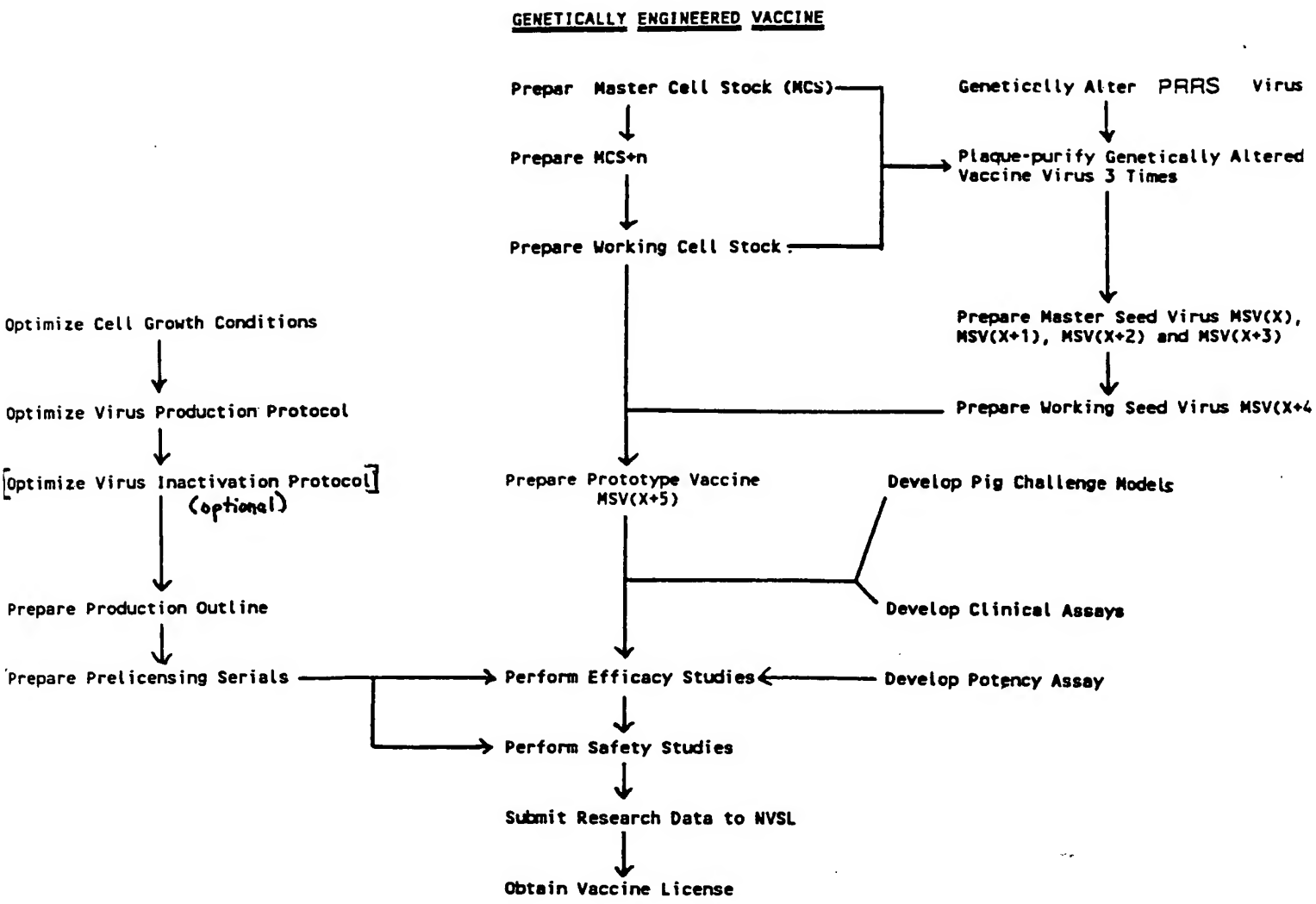

GENETICALLY ENGINEERED VACCINE
Prepar Master Cell Stock (MCS)
Prepare MCS+n
Prepare Working Cell Stock
Geneticcily Alter PRRS Virus
Plaque-purify Genetically Altered Vaccine Virus 3 Times
Prepare Master Seed Virus MSV(X), MSV(X+1), MSV(X+2) and MSV(X+3)
Prepare Working Seed Virus MSV(X+4
Optimize Cell Growth Conditions
Optimize Virus Production Protocol
Optimize Virus Inactivation Protocol
(optional)
Prepare Production Outline
Prepare Prelicensing Serials
Prepare Prototype Vaccine MSV(X+5)
Develop Pig Challenge Models
Develop Clinical Assays
Perform Efficacy Studies
Develop Potency Assay
Perform Safety Studies
Submit Research Data to NVSL
Obtain Vaccine License

FIGURE 2

ISU-12 cDNA λ Library Construction

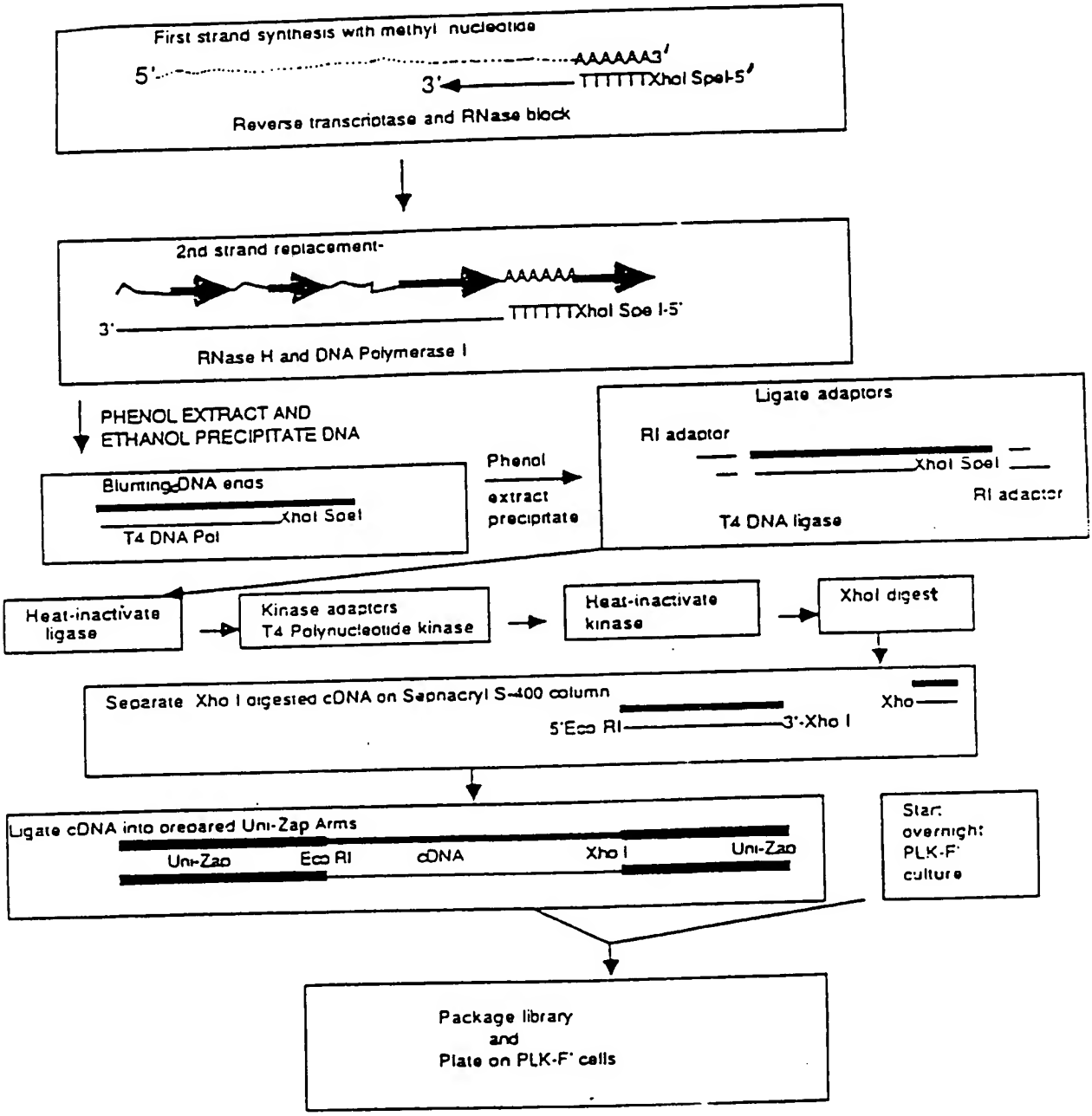


FIGURE 3

Identification of ISU-12 Authentic Clones by Differential Hybridization

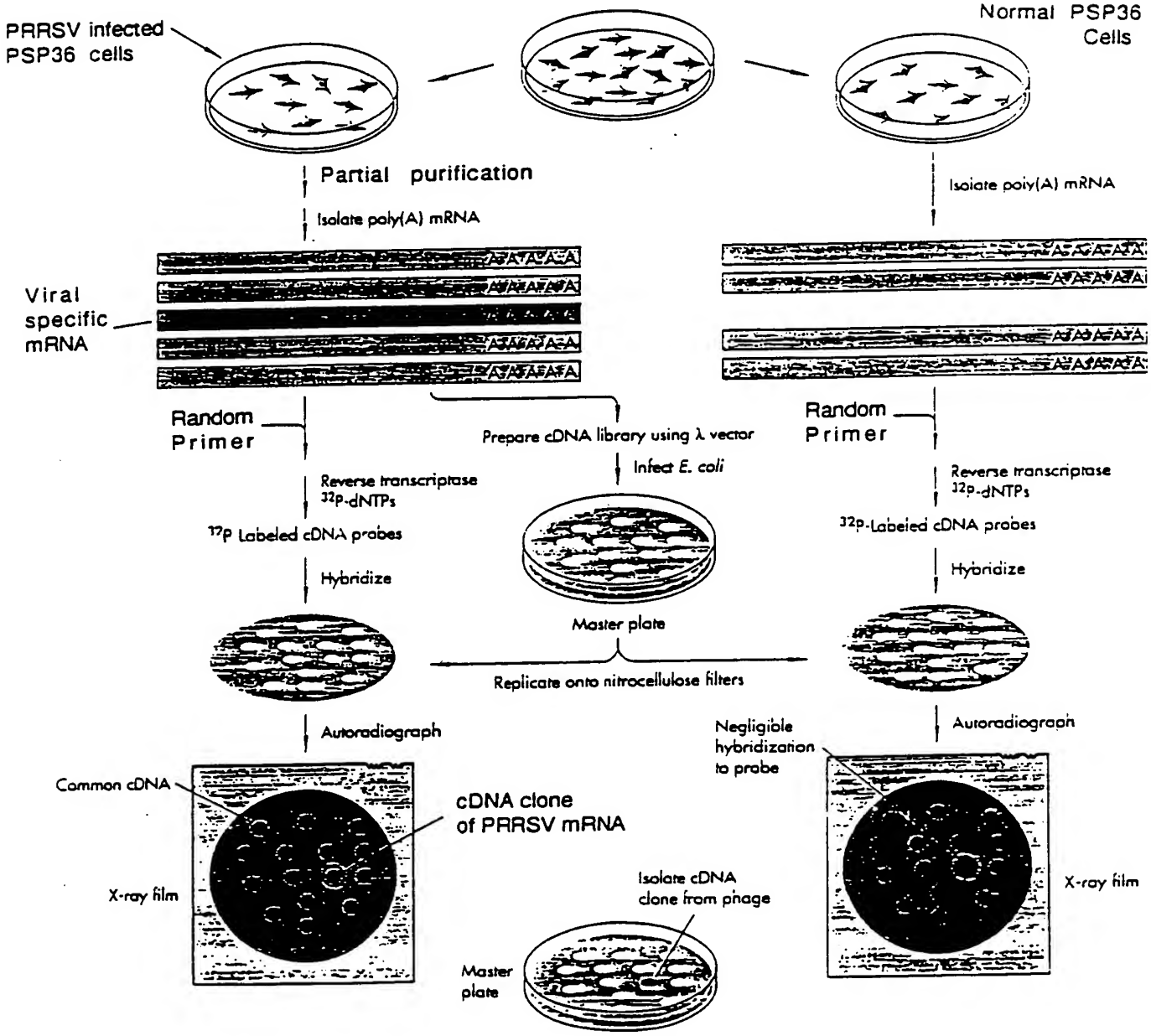


FIGURE 4

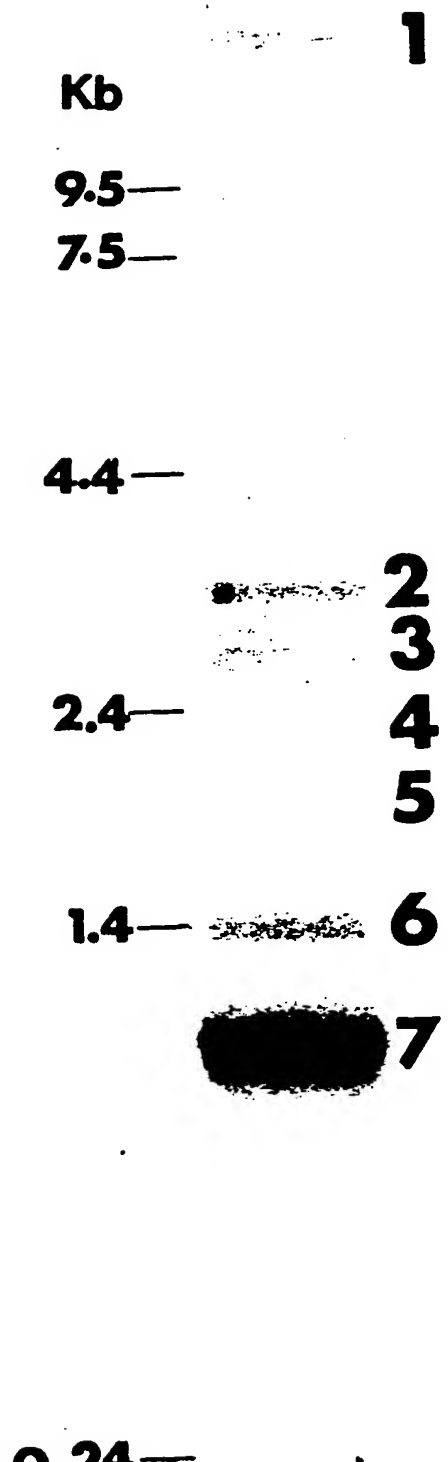


FIGURE 5

ISU-12-7a 3' terminal Graphics

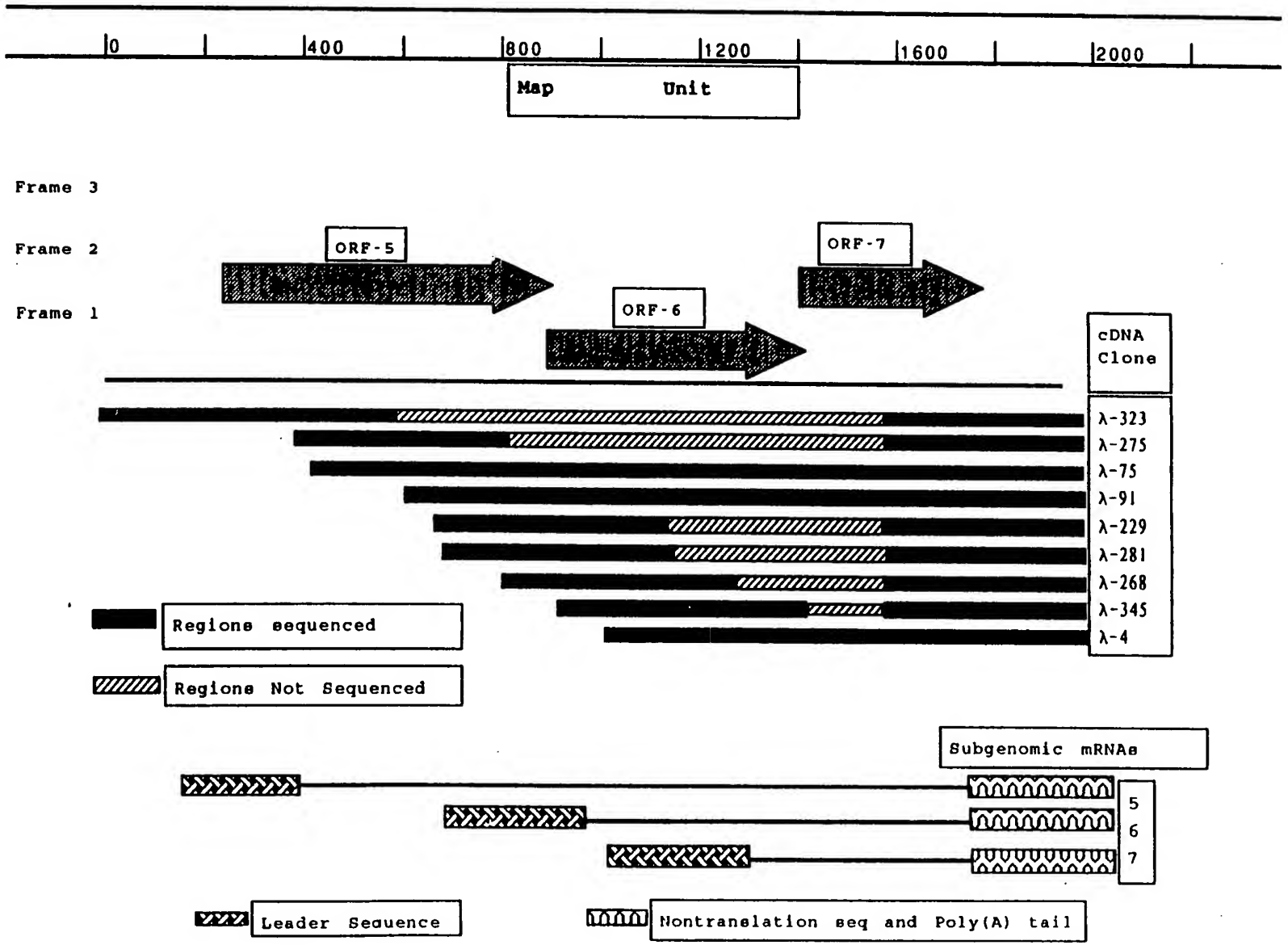
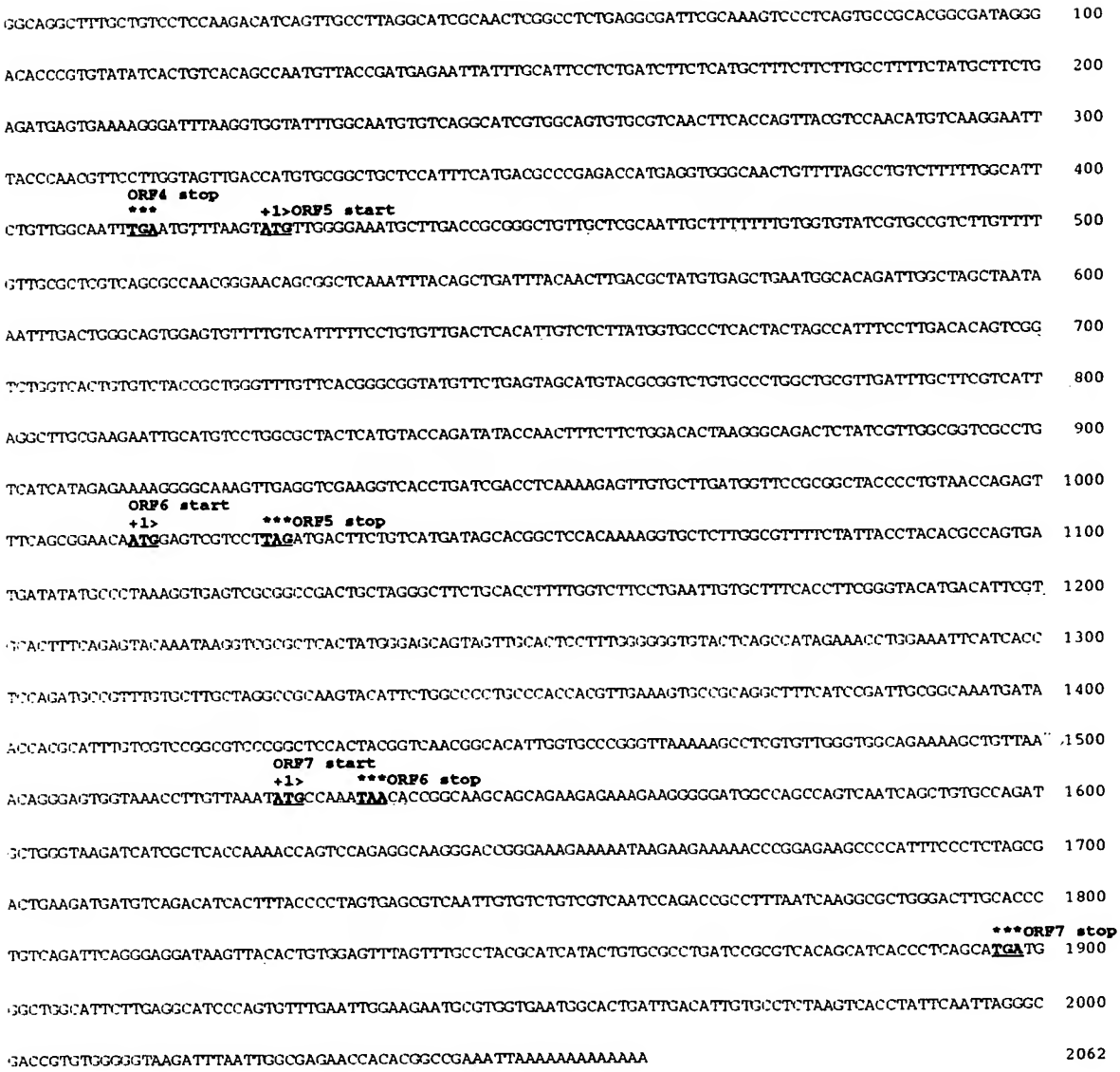


FIGURE 6

FIGURE 7


ORF4 stop
***
+1>ORF5 start
ORF6 start
+1>
***ORF5 stop
ORF7 start
+1>
***ORF6 stop
***ORF7 stop

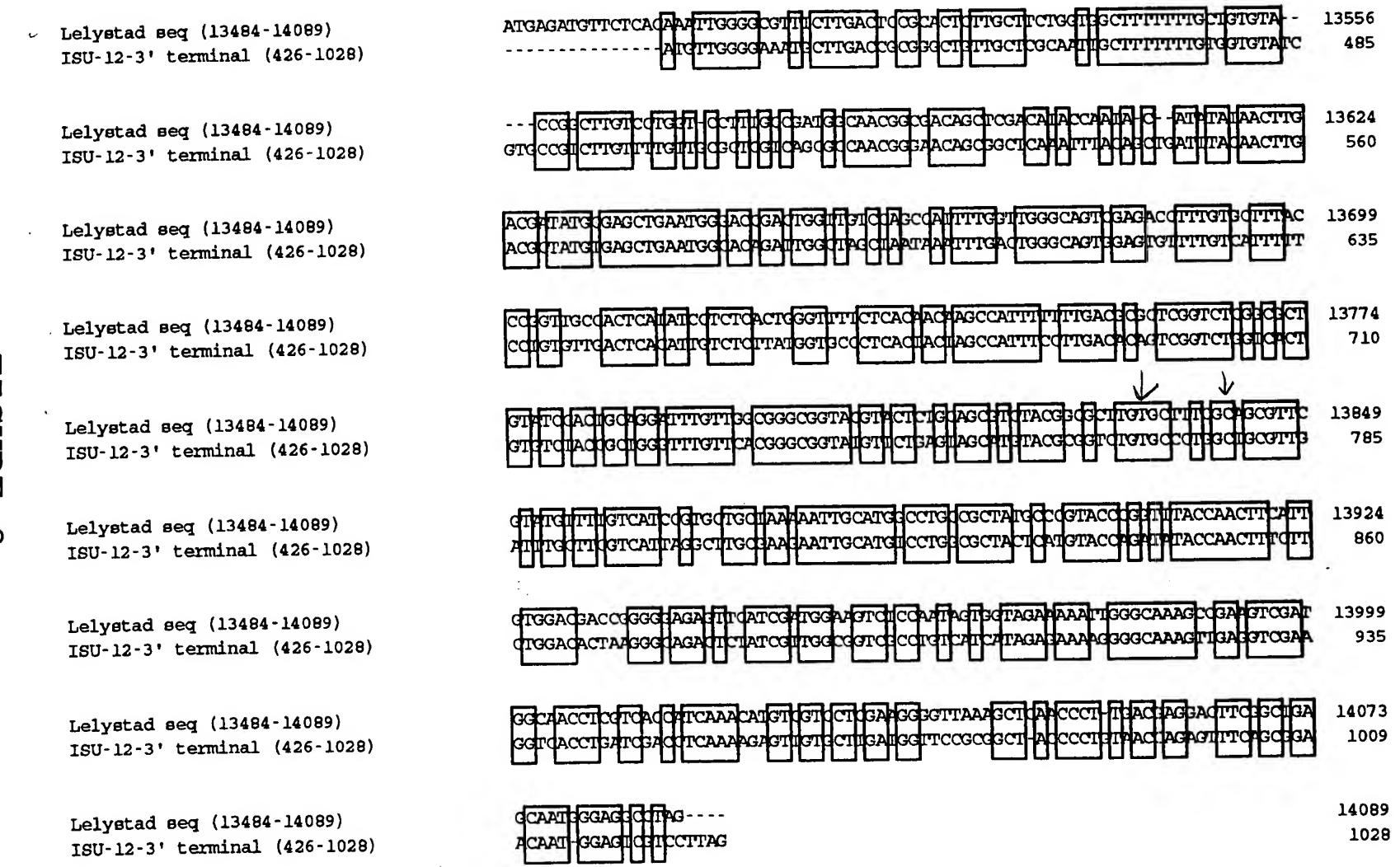

Lelystad seq (13484-14089)
ISU-12-3' terminal (426-1028)
Lelystad seq (13484-14089)
ISU-12-3' terminal (426-1028)
Lelystad seq (13484-14089)
ISU-12-3' terminal (426-1028)
Lelystad seq (13484-14089)
ISU-12-3' terminal (426-1028)
Lelystad seq (13484-14089)
ISU-12-3' terminal (426-1028)
Lelystad seq (13484-14089)
ISU-12-3' terminal (426-1028)
Lelystad seq (13484-14089)
ISU-12-3' terminal (426-1028)
Lelystad seq (13484-14089)
ISU-12-3' terminal (426-1028)
Lelystad seq (13484-14089)
ISU-12-3' terminal (426-1028)
13556
485
13624
560
13699
635
13774
710
13849
785
13924
860
13999
935
14073
1009
14089
1028

FIGURE 8

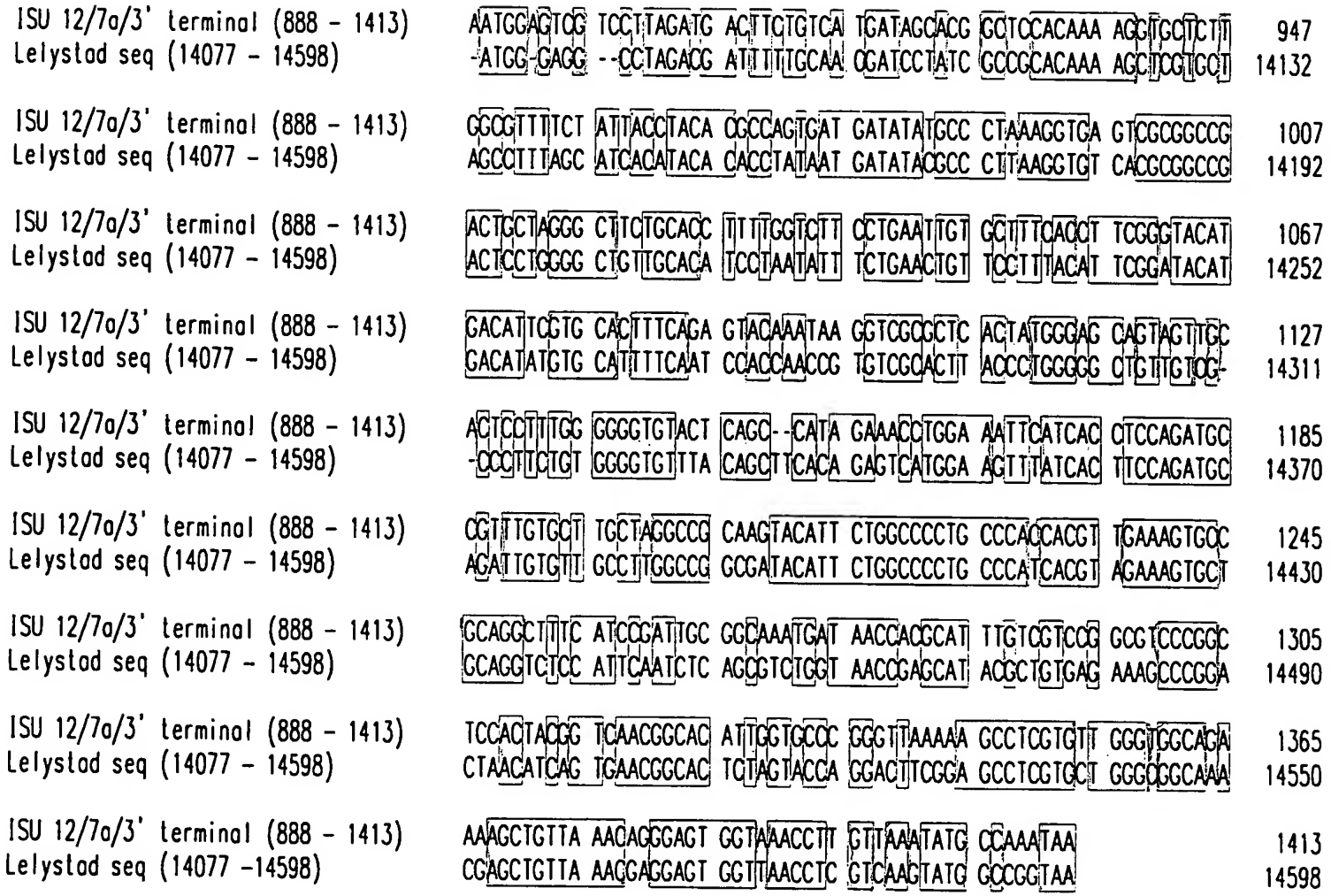

ISU 12/7a/3' terminal (888 - 1413)
Lelystad seq (14077 - 14598)
AATGGAGTCG TCCTTAGATG ACTTCTGTCA TGATAGCACG GCTCCACAAA AGGTGCTCTT 947
-ATGG-GAGG --CCTAGACG ATTTTTGCAA CGATCCTATC GCCGCACAAA AGCTCGTGCT 14132
ISU 12/7a/3' terminal (888 - 1413)
Lelystad seq (14077 - 14598)
GGCGTTTTCT ATTACCTACA CGCCAGTGAT GATATATGCC CTAAAGGTCA GTCGCGGCCG 1007
AGCCTTTAGC ATCACATACA CACCTATAAT GATATACGCC CTTAAGGTGT CACGCGGCCG 14192
ISU 12/7a/3' terminal (888 - 1413)
Lelystad seq (14077 - 14598)
ACTGCTAGGG CTTCTGCACC TTTTGGTCTT CCTGAATTGT GCTTTCACCT TCGGGTACAT 1067
ACTCCTGGGG CTGTTGCACA TCCTAATATT TCTGAACTGT TCCTTTACAT TCGGATACAT 14252
ISU 12/7a/3' terminal (888 - 1413)
Lelystad seq (14077 - 14598)
GACATTCGTG CACTTTCAGA GTACAAATAA GGTCGCGCTC ACTATGGGAG CAGTAGTTGC 1127
GACATATGTG CATTTTCAAT CCACCAACCG TGTCGCACTT ACCCTGGGGG CTGTTGTCG- 14311
ISU 12/7a/3' terminal (888 - 1413)
Lelystad seq (14077 - 14598)
ACTCCTTTGG GGGGTGTACT CAGC--CATA GAAACCTGGA AATTCATCAC CTCCAGATGC 1185
-CCCTTCTGT GGGGTGTTTA CAGCTTCACA GAGTCATGGA AGTTTATCAC TTCCAGATGC 14370
ISU 12/7a/3' terminal (888 - 1413)
Lelystad seq (14077 - 14598)
CGTTTGTGCT TGCTAGGCCG CAAGTACATT CTGGCCCCTG CCCACGCACGT TGAAAGTGCC 1245
AGATTGTGTT GCCTTGGCCG GCGATACATT CTGGCCCCTG CCCATCACGT AGAAAGTGCT 14430
ISU 12/7a/3' terminal (888 - 1413)
Lelystad seq (14077 - 14598)
GCAGGCTTTC ATCCGATTGC GGCAAATGAT AACCACGCAT TTGTCGTCCG GCGTCCCGGC 1305
GCAGGTCTCC ATTCAATCTC AGCGTCTGGT AACCGAGCAT ACGCTGTGAG AAAGCCCGGA 14490
ISU 12/7a/3' terminal (888 - 1413)
Lelystad seq (14077 - 14598)
TCCACTACGG TCAACGGCAC ATTGGTGCCC GGGTTAAAAA GCCTCGTGTT GGGTGGCAGA 1365
CTAACATCAG TGAACGGCAC TGTAGTACCA GGACTTCGGA GCCTCGTGCT GGGCGGCAAA 14550
ISU 12/7a/3' terminal (888 - 1413)
Lelystad seq (14077 -14598)
AAAGCTGTTA AACAGGGAGT GGTAAACCTT GTTAAATATG CCAAATAA 1413
CGAGCTGTTA AAGGAGGAGT GGTTAACCTC GTCAAGTATG GCCGGTAA 14598

FIGURE 9

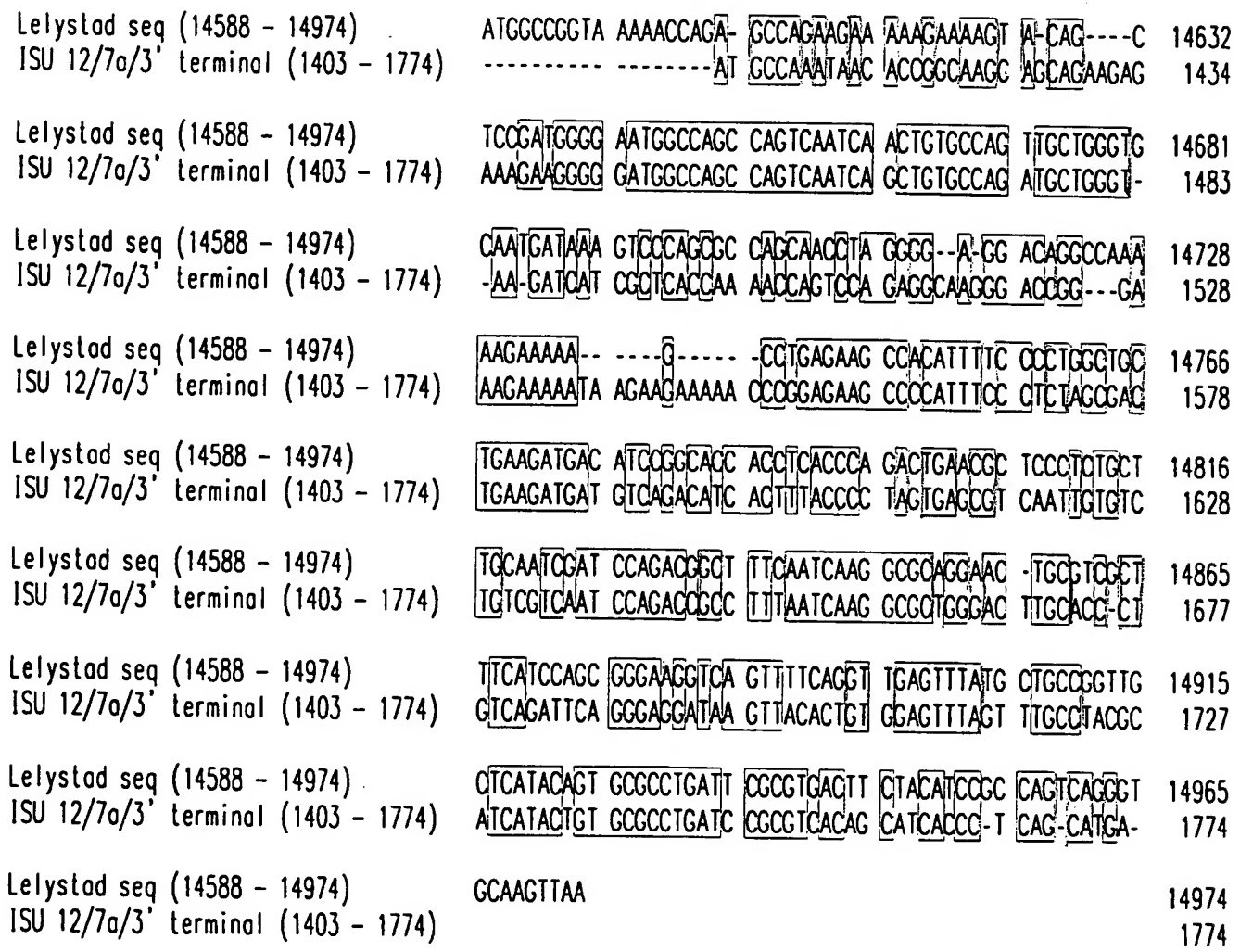

Lelystad seq (14588 - 14974) ATGGCCGGTA AAAACCAGA- GCCAGAAGAA AAAGAAAAGT A-CAG----C 14632
ISU 12/7a/3' terminal (1403 - 1774) ---------- --------AT GCCAAATAAC ACCGGCAAGC AGCAGAAGAG 1434
Lelystad seq (14588 - 14974) TCCGATGGGG AATGGCCAGC CAGTCAATCA ACTGTGCCAG TTGCTGGGTG 14681
ISU 12/7a/3' terminal (1403 - 1774) AAAGAAGGGG GATGGCCAGC CAGTCAATCA GCTGTGCCAG ATGCTGGGT- 1483
Lelystad seq (14588 - 14974) CAATGATAAA GTCCCAGCGC CAGCAACCTA GGGG--A-GG ACAGGCCAAA 14728
ISU 12/7a/3' terminal (1403 - 1774) -AA-GATCAT CGCTCACCAA AACCAGTCCA GACGCAAGGG ACCGG---GA 1528
Lelystad seq (14588 - 14974) AAGAAAAA-- ----G----- -CCTGAGAAG CCACATTTTC CCCTGGCTGC 14766
ISU 12/7a/3' terminal (1403 - 1774) AAGAAAAATA AGAAGAAAAA CCCGGAGAAG CCCCATTTCC CTCTAGCGAC 1578
Lelystad seq (14588 - 14974) TGAAGATGAC ATCCGGCAGC ACCTCACCCA GACTGAACGC TCCCTCTGCT 14816
ISU 12/7a/3' terminal (1403 - 1774) TGAAGATGAT GTCAGACATC ACTTTACCCC TAGTGAGCGT CAATTGTGTC 1628
Lelystad seq (14588 - 14974) TGCAATCGAT CCAGACGGCT TTCAATCAAG GCGCAGGAAC -TGCGTCGCT 14865
ISU 12/7a/3' terminal (1403 - 1774) TGTCGTCAAT CCAGACGCCC TTTAATCAAG GCGCTGGGAC TTGCACC-CT 1677
Lelystad seq (14588 - 14974) TTCATCCAGC GCGAAGGTCA GTTTTCAGGT TGAGTTTATG CTGCCGGTTG 14915
ISU 12/7a/3' terminal (1403 - 1774) GTCAGATTCA GGCAGGATAA GTTACACTGT GGAGTTTAGT TTGCCTACGC 1727
Lelystad seq (14588 - 14974) CTCATACAGT GCGCCTGATT CGCGTGACTT CTACATCCGC CAGTCAGGGT 14965
ISU 12/7a/3' terminal (1403 - 1774) ATCATACTGT GCGCCTGATC CGCGTCACAG CATCACCC-T CAG-CATGA- 1774
Lelystad seq (14588 - 14974) GCAAGTTAA 14974
ISU 12/7a/3' terminal (1403 - 1774) 1774

FIGURE 10

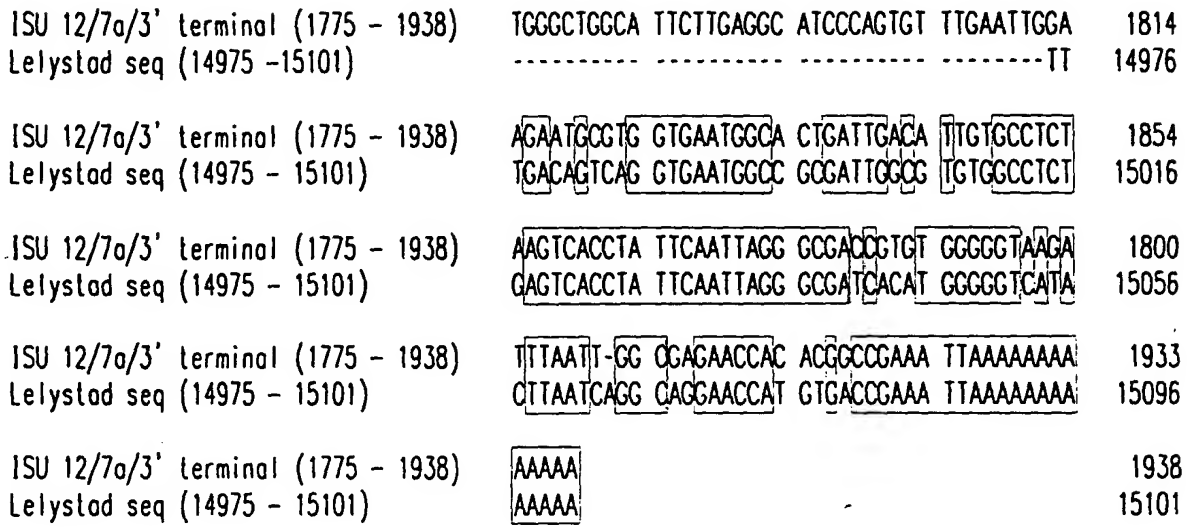

ISU 12/7a/3' terminal (1775 - 1938) TGGGCTGGCA TTCTTGAGGC ATCCCAGTGT TTGAATTGGA 1814
Lelystad seq (14975 -15101) ---------- ---------- ---------- --------TT 14976
ISU 12/7a/3' terminal (1775 - 1938) AGAATGCGTG GTGAATGGCA CTGATTGACA TTGTGCCTCT 1854
Lelystad seq (14975 - 15101) TGACAGTCAG GTGAATGGCC GCGATTGGCG TGTGGCCTCT 15016
ISU 12/7a/3' terminal (1775 - 1938) AAGTCACCTA TTCAATTAGG GCGACCGTGT GGGGGTAAGA 1800
Lelystad seq (14975 - 15101) GAGTCACCTA TTCAATTAGG GCGATCACAT GGGGGTCATA 15056
ISU 12/7a/3' terminal (1775 - 1938) TTTAATT-GG CGAGAACCAC ACGGCCGAAA TTAAAAAAAA 1933
Lelystad seq (14975 - 15101) CTTAATCAGG CAGGAACCAT GTGACCGAAA TTAAAAAAAA 15096
ISU 12/7a/3' terminal (1775 - 1938) AAAAA 1938
Lelystad seq (14975 - 15101) AAAAA 15101

FIGURE 11

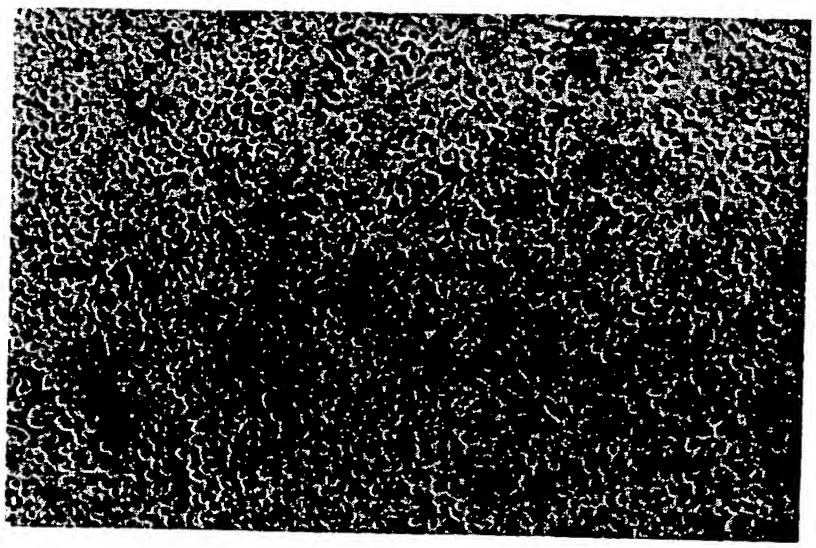


FIGURE 12

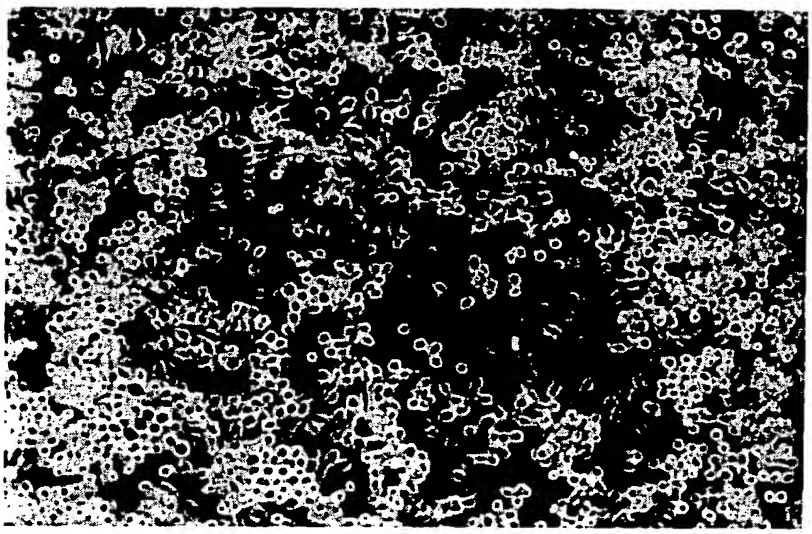


FIGURE 13

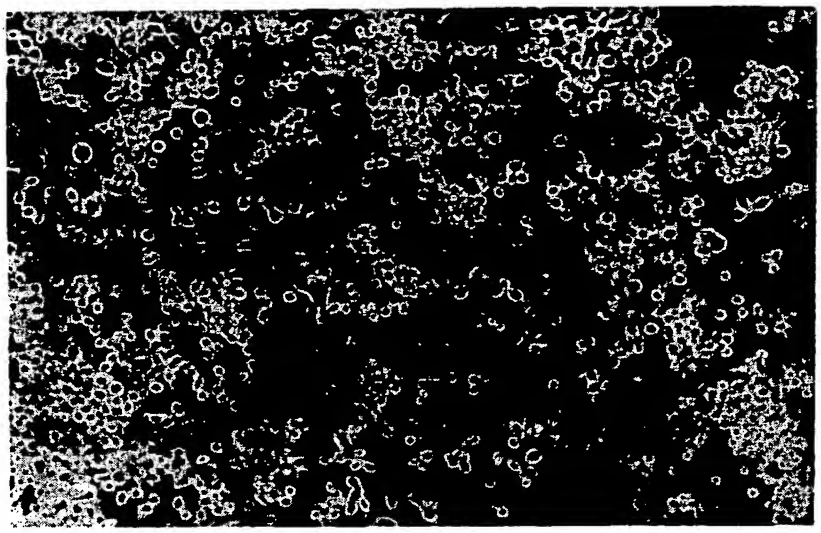


FIGURE 14

FIGURE 15

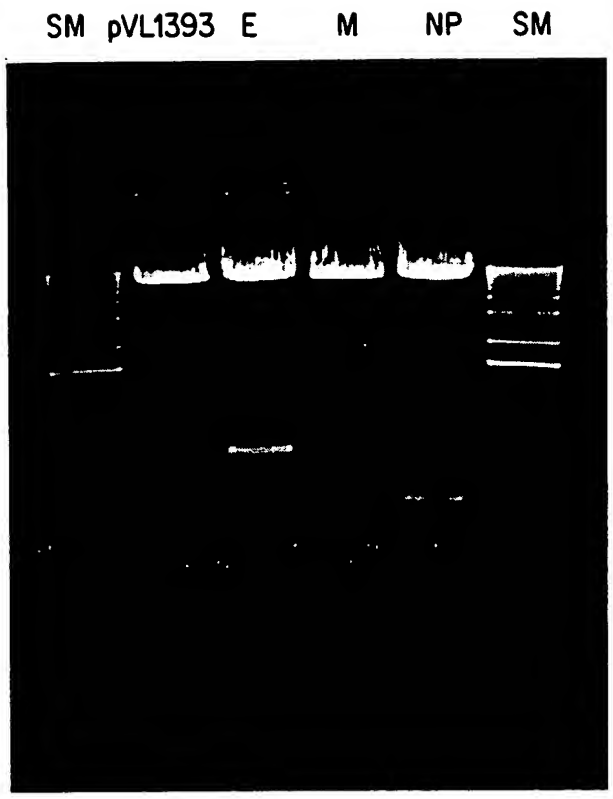
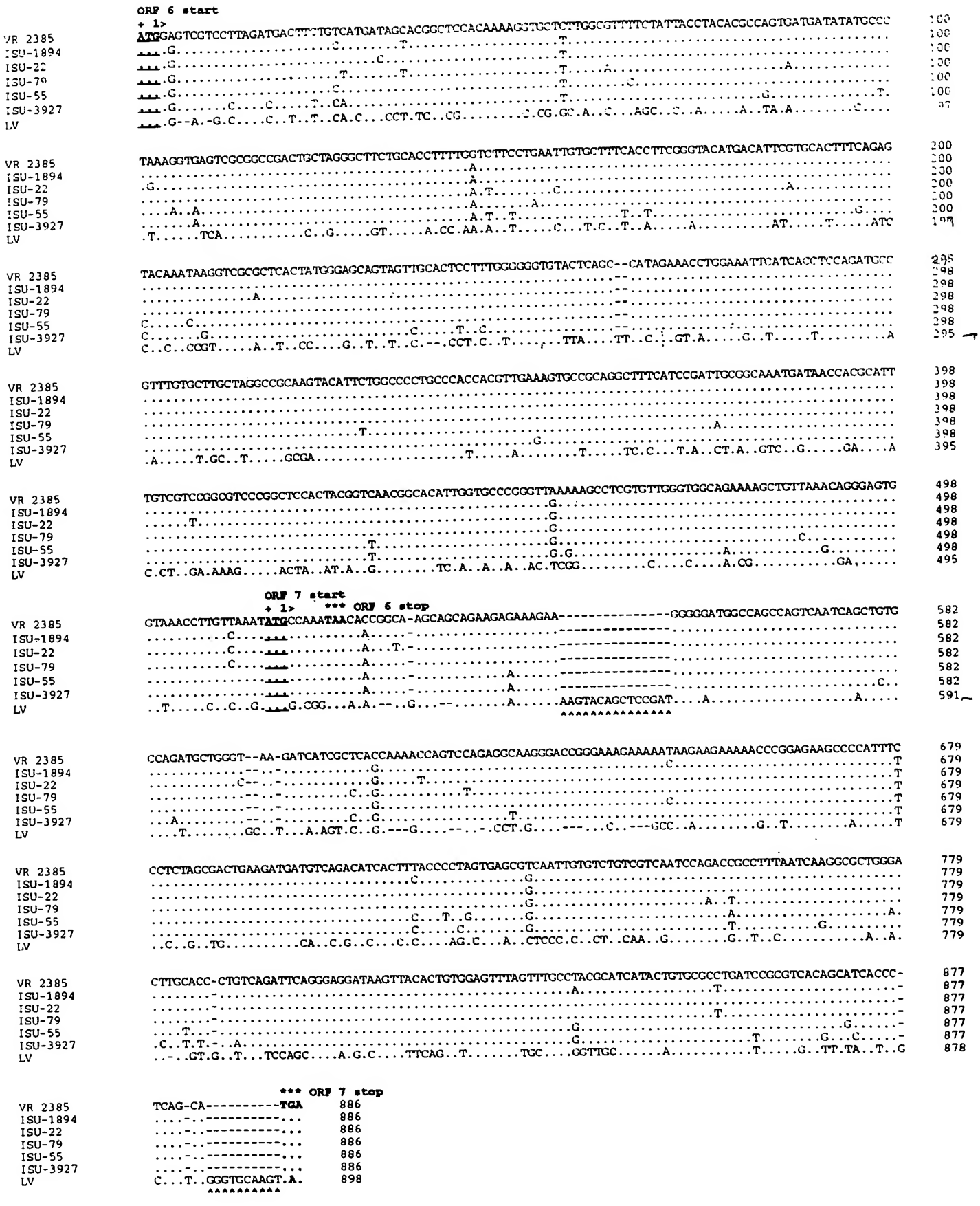


FIGURE 16

FIGURE 17


ORF 6 start
+ 1>
VR 2385
ISU-1894
ISU-22
ISU-79
ISU-55
ISU-3927
LV
ORF 7 start
+ 1>
*** ORF 6 stop
*** ORF 7 stop

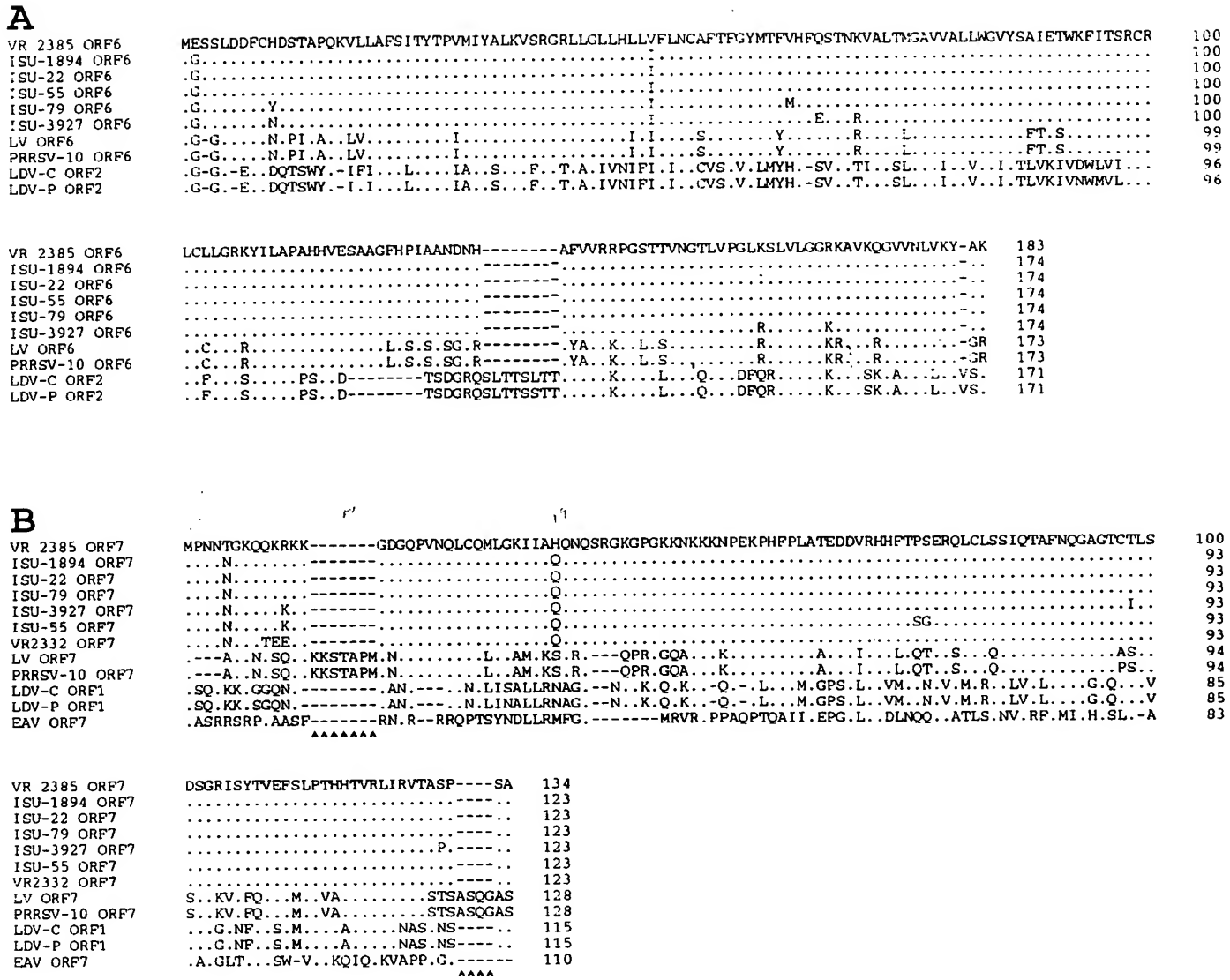
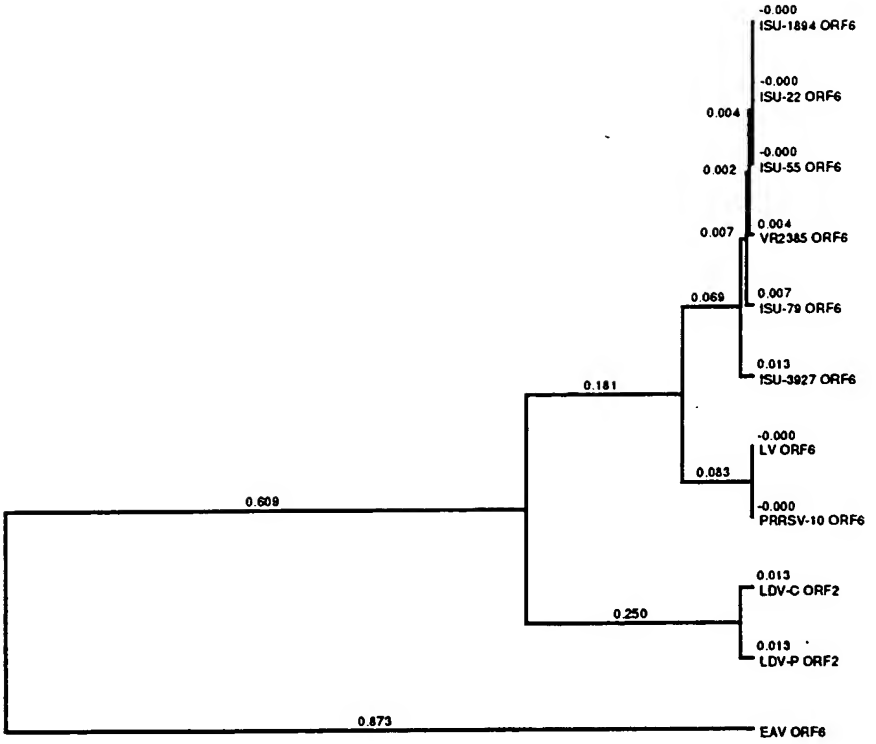

A
VR 2385 ORF6
ISU-1894 ORF6
ISU-22 ORF6
ISU-55 ORF6
ISU-79 ORF6
ISU-3927 ORF6
LV ORF6
PRRSV-10 ORF6
LDV-C ORF2
LDV-P ORF2
B
VR 2385 ORF7
ISU-1894 ORF7
ISU-22 ORF7
ISU-79 ORF7
ISU-3927 ORF7
ISU-55 ORF7
VR2332 ORF7
LV ORF7
PRRSV-10 ORF7
LDV-C ORF1
LDV-P ORF1
EAV ORF7

FIGURE 18

A


-0.000
ISU-1894 ORF6
-0.000
ISU-22 ORF6
0.004
-0.000
ISU-55 ORF6
0.002
0.004
VR2385 ORF6
0.007
0.007
ISU-79 ORF6
0.069
0.013
ISU-3927 ORF6
0.181
-0.000
LV ORF6
0.083
-0.000
PRRSV-10 ORF6
0.609
0.013
LDV-C ORF2
0.250
0.013
LDV-P ORF2
0.873
EAV ORF6

B

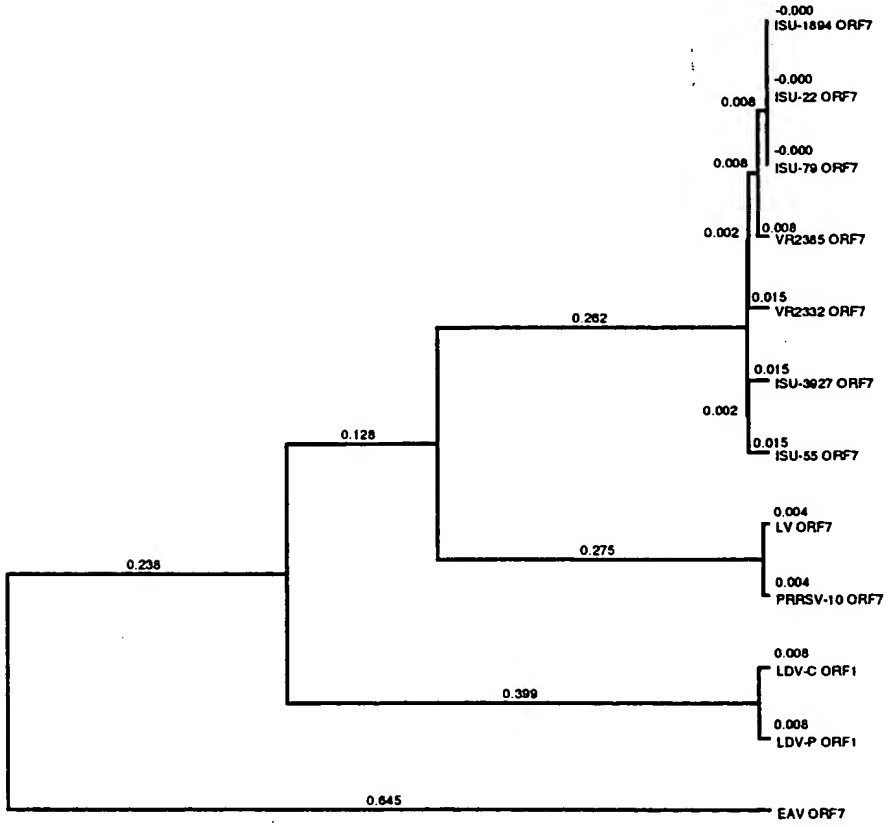

-0.000
ISU-1894 ORF7
-0.000
ISU-22 ORF7
0.008
-0.000
ISU-79 ORF7
0.008
0.008
VR2385 ORF7
0.002
0.015
VR2332 ORF7
0.262
0.015
ISU-3927 ORF7
0.002
0.015
ISU-55 ORF7
0.128
0.004
LV ORF7
0.275
0.004
PRRSV-10 ORF7
0.238
0.008
LDV-C ORF1
0.399
0.008
LDV-P ORF1
0.645
EAV ORF7

FIGURE 19

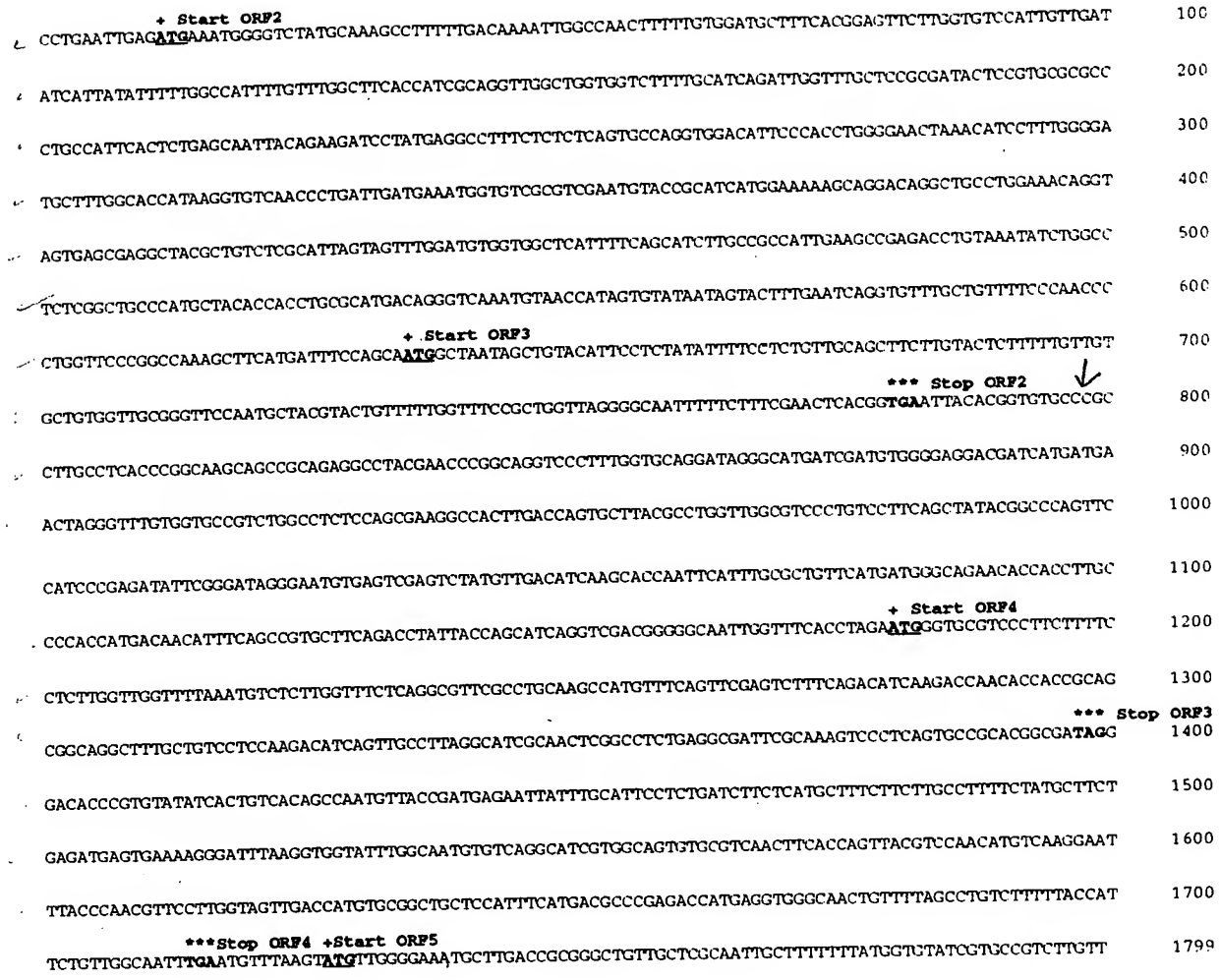

+ Start ORF2
CCTGAATTGAGATGAAATGGGGTCTATGCAAAGCCTTTTTGACAAAATTGGCCAACTTTTTGTGGATGCTTTCACGGAGTTCTTGGTGTCCATTGTTGAT 100
ATCATTATATTTTTGGCCATTTTGTTTGGCTTCACCATCGCAGGTTGGCTGGTGGTCTTTTTGCATCAGATTGGTTTGCTCCGCGATACTCCGTGCGCGCC 200
CTGCCATTCACTCTGAGCAATTACAGAAGATCCTATGAGGCCTTTCTCTCTCAGTGCCAGGTGGACATTCCCACCTGGGGAACTAAACATCCTTTGGGGA 300
TGCTTTGGCACCATAAGGTGTCAACCCTGATTGATGAAATGGTGTCGCGTCGAATGTACCGCATCATGGAAAAAGCAGGACAGGCTGCCTGGAAACAGGT 400
AGTGAGCGAGGCTACGCTGTCTCGCATTAGTAGTTTGGATGTGGTGGCTCATTTTCAGCATCTTGCCGCCATTGAAGCCGAGACCTGTAAATATCTGGCC 500
TCTCGGCTGCCCATGCTACACCACCTGCGCATGACAGGGTCAAATGTAACCATAGTGTATAATAGTACTTTGAATCAGGTGTTTGCTGTTTTCCCAACCC 600
+ Start ORF3
CTGGTTCCCGGCCAAAGCTTCATGATTTCCAGCAATGGCTAATAGCTGTACATTCCTCTATATTTTCCTCTGTTGCAGCTTCTTGTACTCTTTTTGTTGT 700
*** Stop ORF2
GCTGTGGTTGCGGGTTCCAATGCTACGTACTGTTTTTGGTTTCCGCTGGTTAGGGGCAATTTTTCTTTCGAACTCACGGTGAATTACACGGTGTGCCCGC 800
CTTGCCTCACCCGGCAAGCAGCCGCAGAGGCCTACGAACCCGGCAGGTCCCTTTGGTGCAGGATAGGGCATGATCGATGTGGGGAGGACGATCATGATGA 900
ACTAGGGTTTGTGGTGCCGTCTGGCCTCTCCAGCGAAGGCCACTTGACCAGTGCTTACGCCTGGTTGGCGTCCCTGTCCTTCAGCTATACGGCCCAGTTC 1000
CATCCCGAGATATTCGGGATAGGGAATGTGAGTCGAGTCTATGTTGACATCAAGCACCAATTCATTTGCGCTGTTCATGATGGGCAGAACACCACCTTGC 1100
+ Start ORF4
CCCACCATGACAACATTTCAGCCGTGCTTCAGACCTATTACCAGCATCAGGTCGACGGGGGCAATTGGTTTCACCTAGAATGGGTGCGTCCCTTCTTTTC 1200
CTCTTGGTTGGTTTTAAATGTCTCTTGGTTTCTCAGGCGTTCGCCTGCAAGCCATGTTTCAGTTCGAGTCTTTCAGACATCAAGACCAACACCACCGCAG 1300
*** Stop ORF3
CGGCAGGCTTTGCTGTCCTCCAAGACATCAGTTGCCTTAGGCATCGCAACTCGGCCTCTGAGGCGATTCGCAAAGTCCCTCAGTGCCGCACGGCGATAGG 1400
GACACCCGTGTATATCACTGTCACAGCCAATGTTACCGATGAGAATTATTTGCATTCCTCTGATCTTCTCATGCTTTCTTCTTGCCTTTTCTATGCTTCT 1500
GAGATGAGTGAAAAGGGATTTAAGGTGGTATTTGGCAATGTGTCAGGCATCGTGGCAGTGTGCGTCAACTTCACCAGTTACGTCCAACATGTCAAGGAAT 1600
TTACCCAACGTTCCTTGGTAGTTGACCATGTGCGGCTGCTCCATTTCATGACGCCCGAGACCATGAGGTGGGCAACTGTTTTAGCCTGTCTTTTTACCAT 1700
***Stop ORF4 +Start ORF5
TCTGTTGGCAATTTGAATGTTTAAGTATGTTGGGGAAATGCTTGACCGCGGGCTGTTGCTCGCAATTGCTTTTTTTTATGGTGTATCGTGCCGTCTTGTT 1799

FIGURE 20

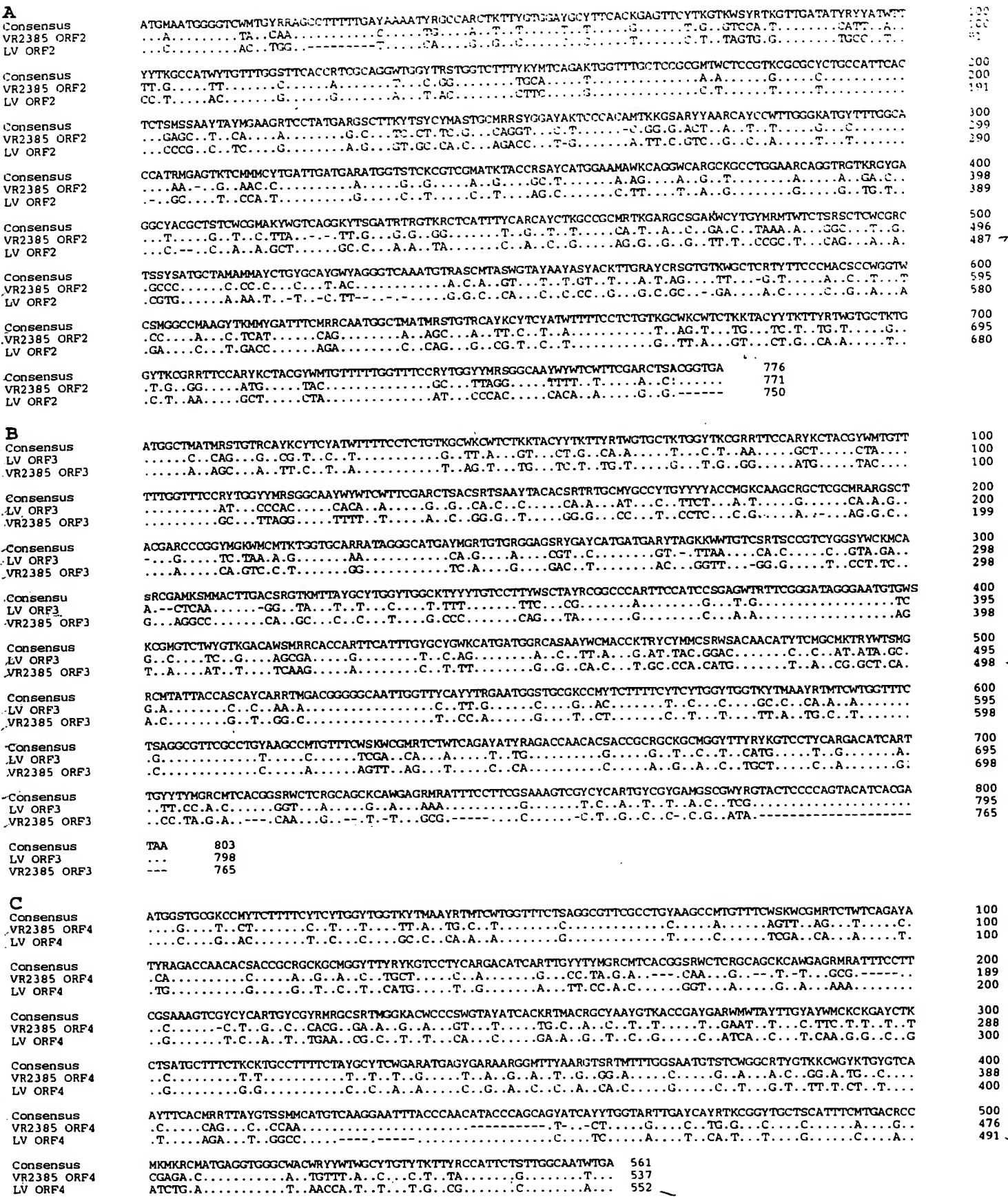
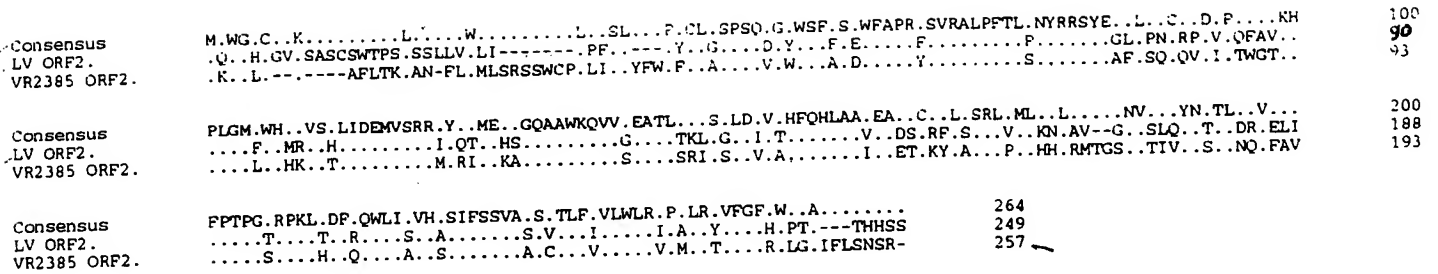
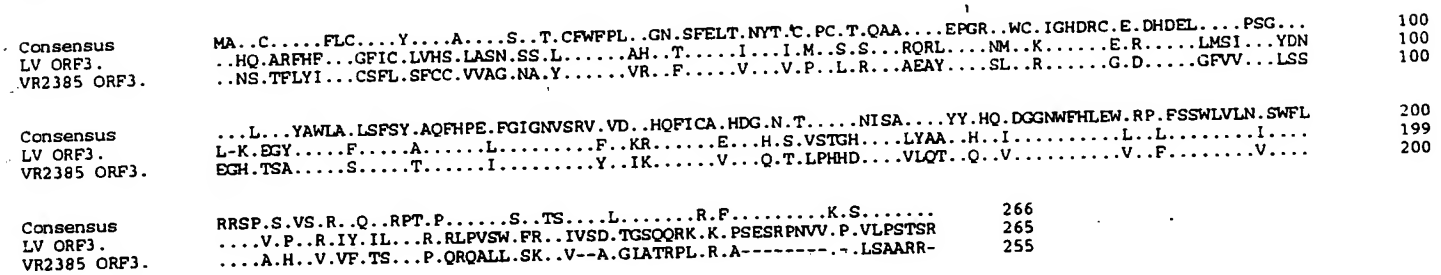

A
Consensus
VR2385 ORF2
LV ORF2
B
Consensus
LV ORF3
VR2385 ORF3
C
Consensus
VR2385 ORF4
LV ORF4

FIGURE 21

A



B



C

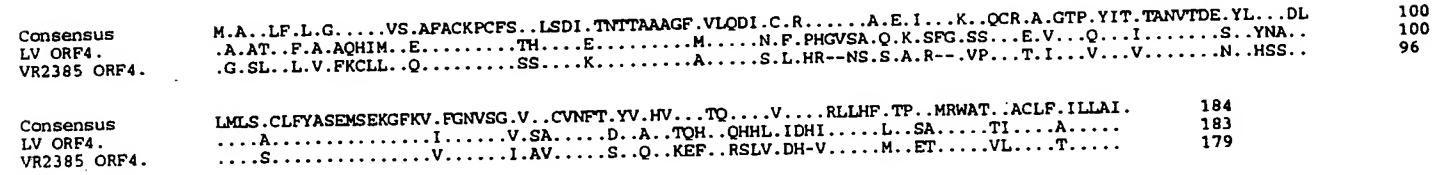


FIGURE 22

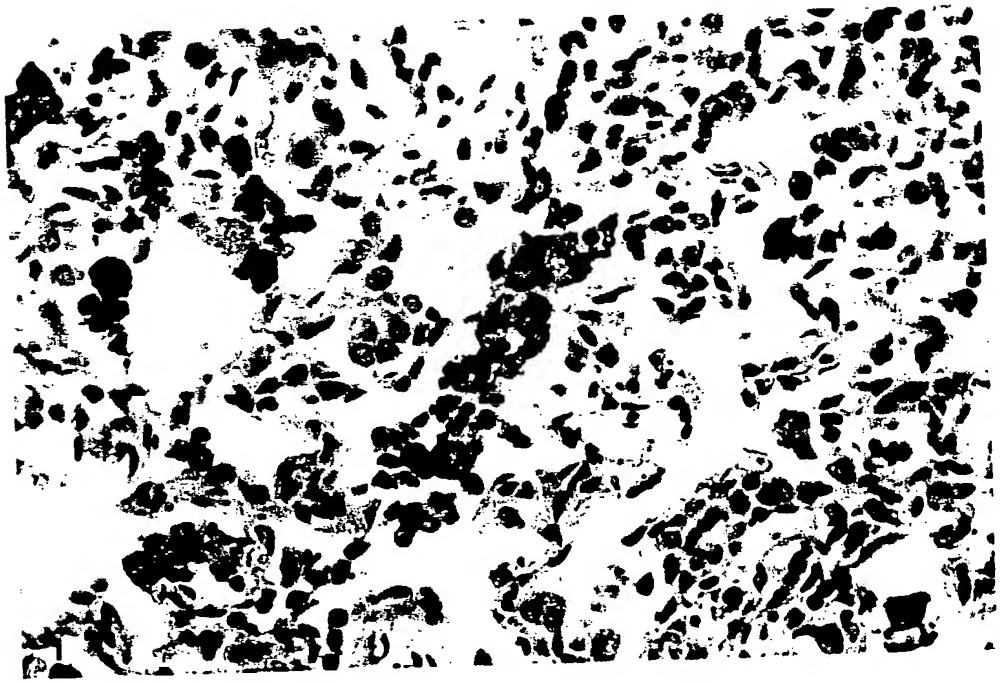


FIGURE 23

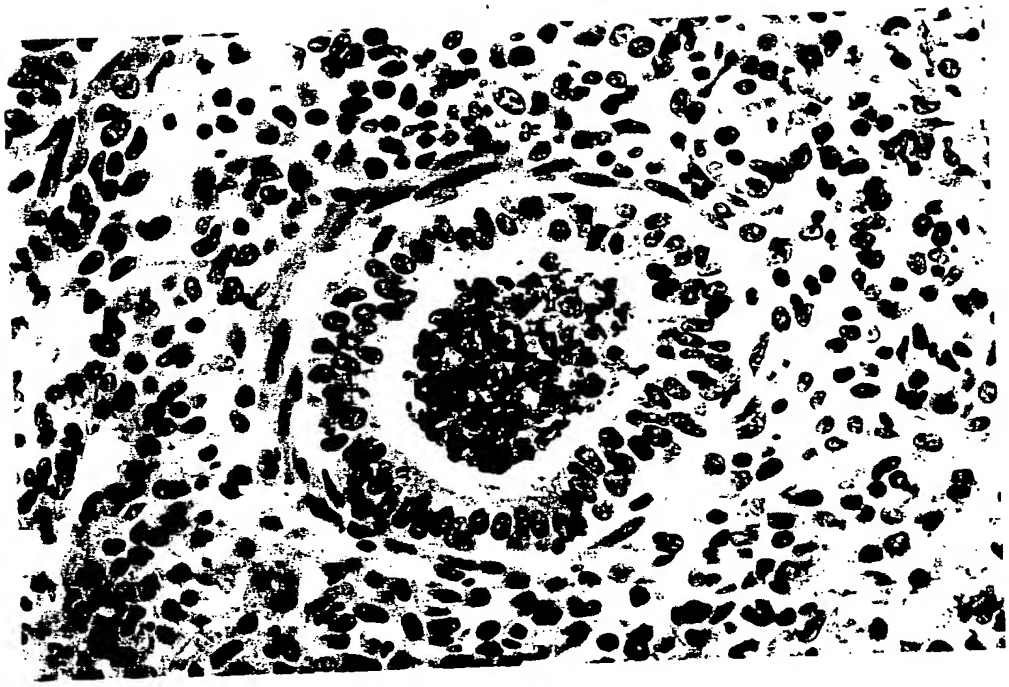


FIGURE 24

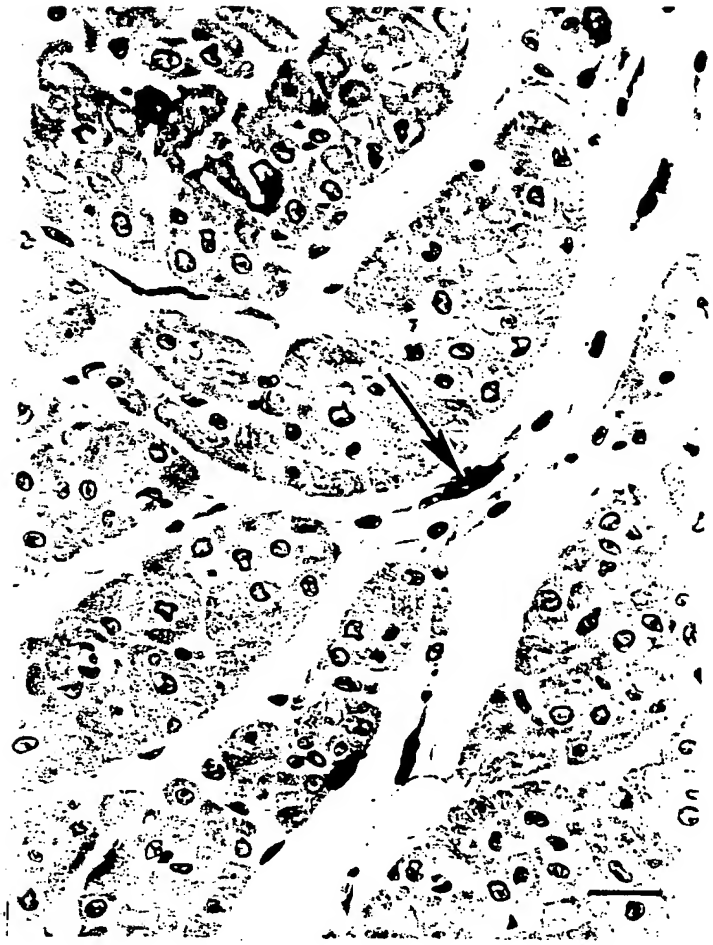


FIGURE 25

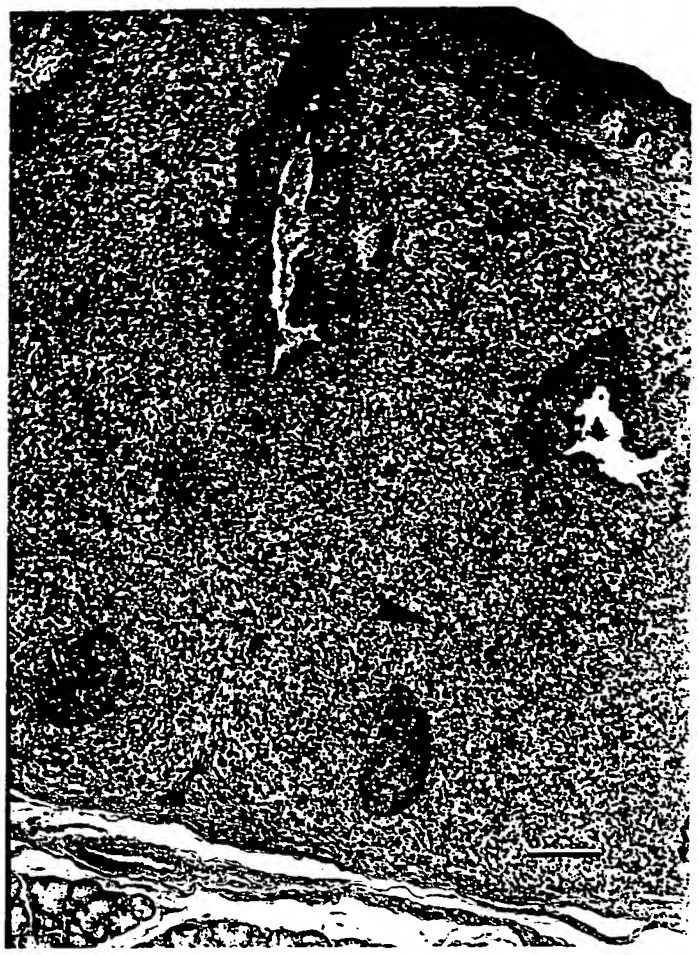


FIGURE 26

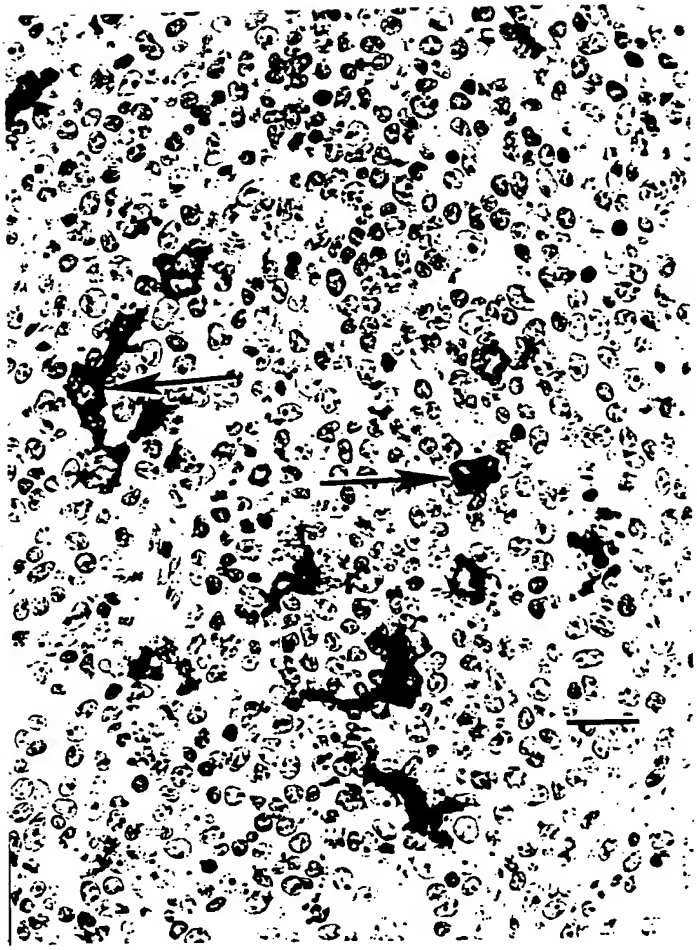


FIGURE 27

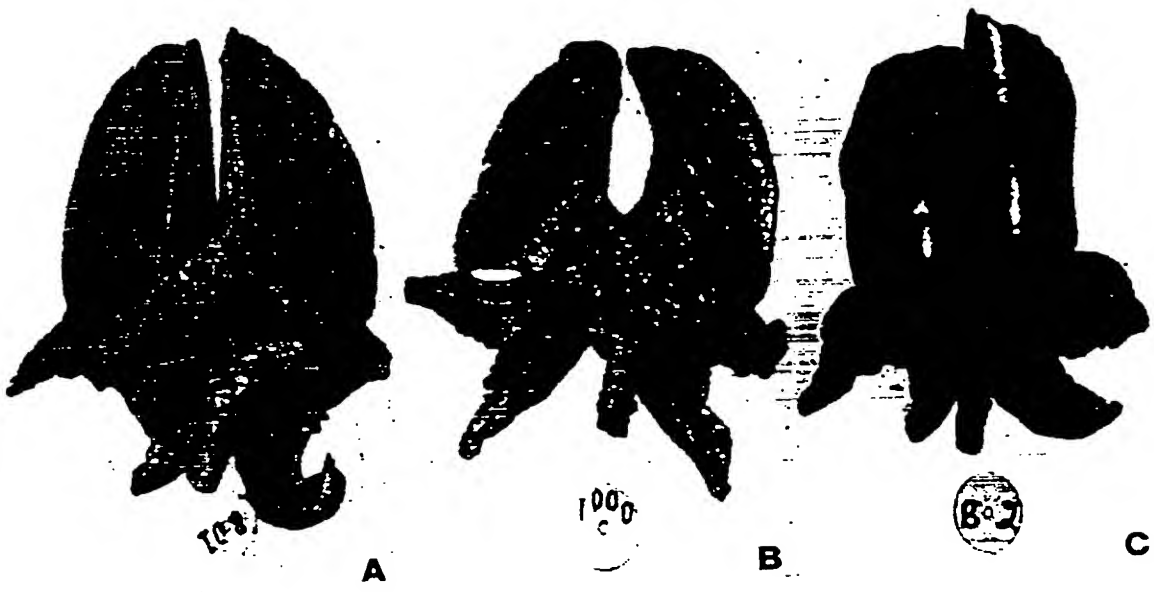


FIGURE 28

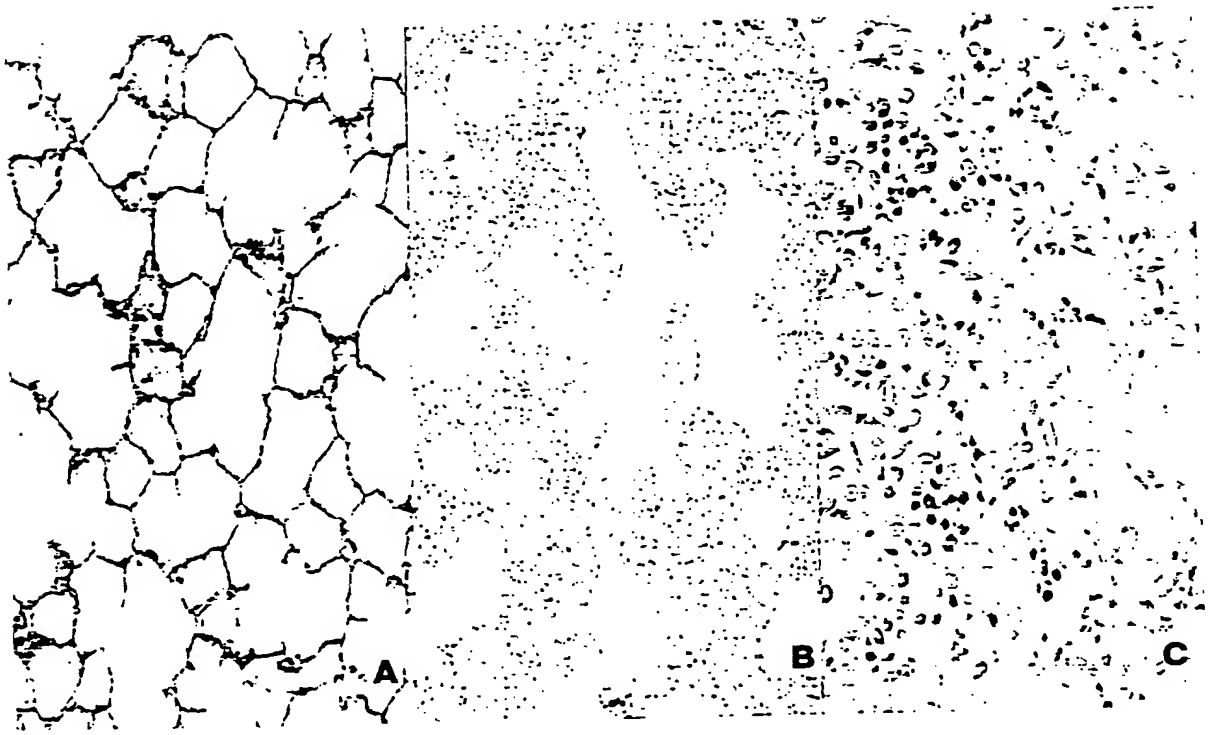


FIGURE 29

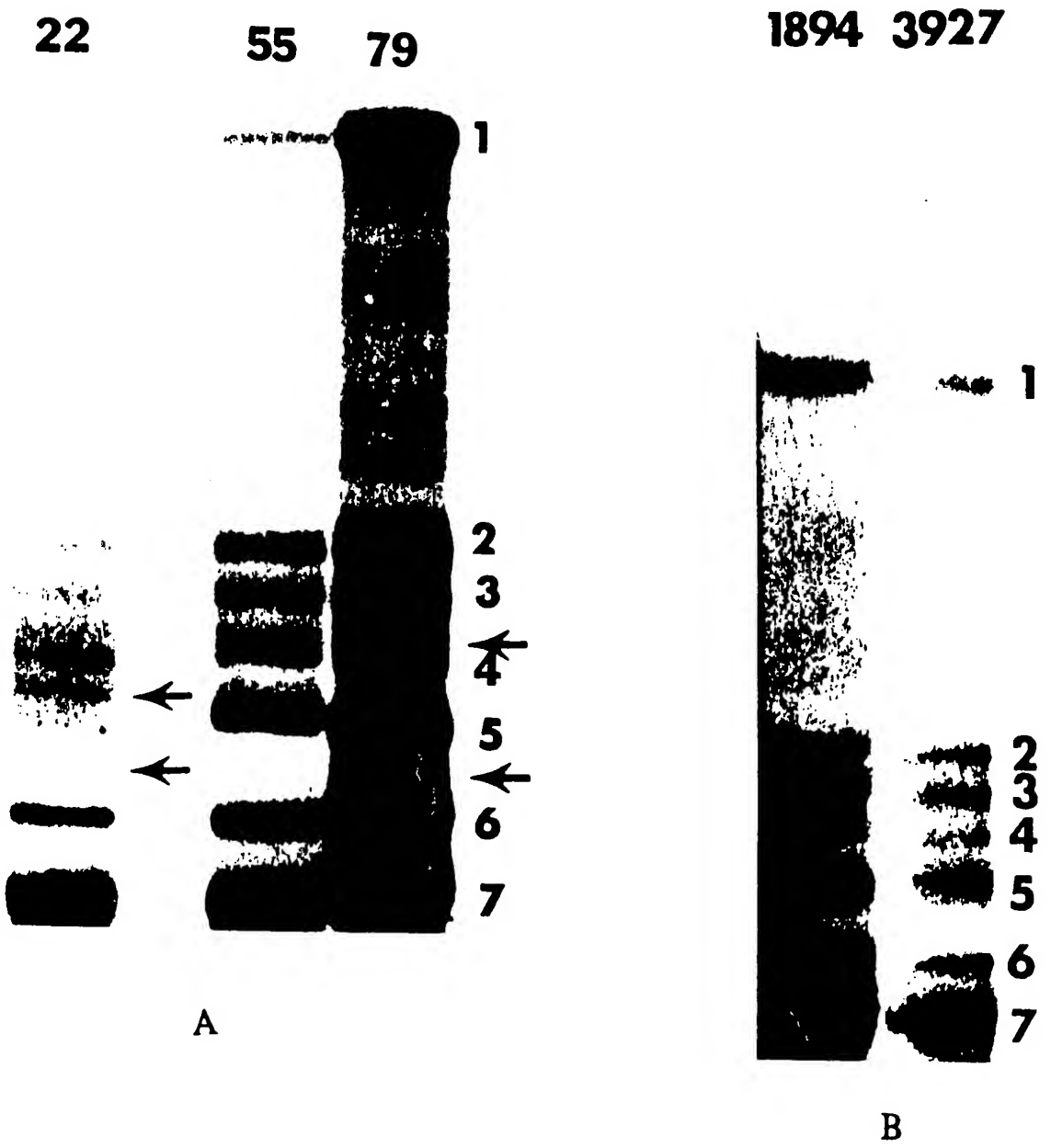


FIGURE 30